

Amendments Claims:

Please amend the claims as follows:

1. (Previously Presented): A method for amplification of a template DNA, comprising:

(a) incubating a reaction mixture, said reaction mixture comprising:

(i) a template DNA:

(ii) a first primer, wherein the first primer is hybridizable to a multiplicity of template sites, wherein the first primer is a population of different composite primers each comprising an RNA portion and a 3' DNA portion, wherein each composite primer comprises a 3' random sequence, and wherein each composite primer is a tailed primer that comprises a 5' portion that is not hybridizable to the template DNA under conditions in which the first primer hybridizes to the template DNA;

(iii) a DNA-dependent DNA polymerase; and

(iv) an RNA-dependent DNA polymerase;

wherein the incubation is under conditions that permit generation of a complex comprising an RNA/DNA heteroduplex, wherein said complex comprising an RNA/DNA heteroduplex is produced by hybridization of the first primer to the template DNA, primer extension to generate a first primer extension product, hybridization of a second primer to the first primer extension product, and extension of the second primer to generate a second primer extension product; and

(b) incubating a reaction mixture, said reaction mixture comprising

(i) at least a portion of the reaction products generated according to step (a);

(ii) an amplification primer, wherein said amplification primer is a composite primer comprising an RNA portion and a 3' DNA portion, wherein the amplification primer comprises some of the sequence of the first primer, and wherein the first primer and the amplification primer are different primers;

(iii) a DNA-dependent DNA polymerase; and

(iv) an agent that cleaves RNA from an RNA/DNA heteroduplex;

wherein the incubation is under conditions that permit RNA cleavage, primer hybridization, primer extension, and displacement of the first primer extension product when its RNA is cleaved and another amplification primer binds to the second primer

extension product and is extended, such that primer extension and strand displacement are repeated, whereby multiple copies of an amplification product are generated.

2. (Previously Presented): The method of claim 1, wherein said DNA-dependent DNA polymerase and said RNA-dependent DNA polymerase of step (a) are the same enzyme.
3. (Previously Presented): The method of claim 1, wherein said DNA-dependent DNA polymerase and said RNA-dependent DNA polymerase of step (a) are different enzymes.
4. (Previously Presented): The method of claim 1, wherein said first primer and said second primer are the same primer.
5. (Previously Presented): The method of claim 1, wherein said first primer and said second primer are different primers.
6. (Original): The method of claim 1, wherein step (b) is initiated by the addition of an agent that cleaves RNA from an RNA/DNA heteroduplex to the reaction mixture of step (a).
7. (Previously Presented): The method of claim 24, wherein said agent that cleaves RNA from an RNA/DNA heteroduplex is RNase H.
- 8.-23. (Canceled).
24. (Previously Presented): The method of claim 1, wherein the agent that cleaves RNA from an RNA/DNA heteroduplex is an enzyme.
- 25.-26. (Canceled).
27. (Previously Presented): The method of claim 1, wherein the RNA portion of the first primer is 5' with respect to the 3' DNA portion.
28. (Previously Presented): The method of claim 27, wherein the RNA portion of the amplification primer is 5' with respect to the 3' DNA portion.
- 29.-32. (Canceled).
33. (Previously Presented): The method of claim 1, wherein the RNA portion of the amplification primer is 5' with respect to the 3' DNA portion.
34. (Original): The method of claim 33, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.
35. (Previously Presented): The method of claim 1, wherein the RNA portion of the first primer consists of about 7 to about 50 nucleotides.

36. (Original): The method of claim 35, wherein the DNA portion of the first primer consists of about 5 to about 20 nucleotides.

37.-38. (Canceled).

39. (Previously Presented): The method of claim 1, wherein the RNA portion of the amplification primer consists of about 7 to about 50 nucleotides.

40. (Original): The method of claim 39, wherein the DNA portion of the amplification primer consists of about 5 to about 20 nucleotides.

41.-45. (Canceled).

46. (Original): The method of claim 1, wherein the reaction mixture of step (b) further comprises a non-canonical nucleotide.

47. (Original): The method of claim 46, wherein the non-canonical nucleotide is dUTP.

48. (Original): The method of claim 1, wherein the reaction mixture of step (b) further comprises a labeled nucleotide.

49. (Currently Amended): A method for amplification of a template DNA, comprising:
incubating a reaction mixture, said reaction mixture comprising:

(a) a complex comprising a RNA/DNA partial heteroduplex, wherein the complex is generated by incubating a first reaction mixture, said first reaction mixture comprising:

(i) a template DNA;

(ii) a first primer; wherein the first primer is hybridizable to a multiplicity of template sites, wherein the first primer is a population of different composite primers each comprising an RNA portion and a 3' DNA portion, wherein the composite primer comprises a 3' random sequence, and wherein the composite primer is a tailed primer that comprises a 5' portion that is not hybridizable to the template DNA under conditions in which the first primer hybridizes to the template DNA;

(iii) a DNA-dependent DNA polymerase; and

(iv) an RNA-dependent DNA polymerase;

wherein the incubation is under conditions that permit hybridization of the first primer to the template DNA, [[.]] primer extension to generate a first primer extension product, hybridization of a second primer to the first primer extension product, and

extension of the second primer to generate a second primer extension product, whereby a complex comprising an RNA/DNA partial heteroduplex is generated;

(b) an amplification primer, wherein the amplification primer is a composite primer comprising an RNA portion and a 3' DNA portion, wherein the amplification primer comprises some of the sequence of the first primer, and wherein the first primer and the amplification primer are different primers;

(c) a DNA-dependent DNA polymerase; and

(d) an agent that cleaves RNA from an RNA/DNA heteroduplex;

wherein the incubation is under conditions that permit RNA cleavage, primer hybridization, primer extension, and displacement of the first primer extension product when its RNA is cleaved and another amplification primer binds to the second primer extension product and is extended, such that primer extension and strand displacement are repeated, whereby multiple copies of an amplification product are generated.

50. (Canceled).

51. (Previously Presented): The method of claim 49, wherein the agent that cleaves RNA from an RNA/DNA heteroduplex is an enzyme.

52. (Previously Presented): The method of claim 51, wherein the enzyme that cleaves RNA from an RNA/DNA heteroduplex is RNase H.

53. (Previously Presented): The method of claim 51, wherein said DNA-dependent DNA polymerase and said enzyme that cleaves RNA from an RNA/DNA heteroduplex are the same enzyme.

54. (Previously Presented): The method of claim 53, wherein said DNA-dependent DNA polymerase and said enzyme that cleaves RNA from an RNA/DNA heteroduplex are different enzymes.

55. (Original): The method of claim 49, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.

56. (Original): The method of claim 55, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.

57. (Previously Presented): The method of claim 49, wherein the RNA portion of the amplification primer consists of about 7 to about 50 nucleotides.

58. (Original): The method of claim 57, wherein the DNA portion of the amplification primer consists of about 5 to about 20 nucleotides.

59. (Previously Presented): The method of claim 49, wherein the RNA portion of the amplification primer consists of about 10 to about 50 nucleotides.

60. (Original): The method of claim 59, wherein the DNA portion of the amplification primer consists of about 7 to about 20 nucleotides.

61. (Canceled).

62. (Original): The method of claim 49, wherein the reaction mixture further comprises a non-canonical nucleotide.

63. (Original): The method of claim 62, wherein the non-canonical nucleotide is dUTP.

64. (Original): The method of claim 49, wherein the reaction mixture further comprises a labeled nucleotide.

65. (Previously Presented): A method for amplification of a template DNA, comprising:
incubating a reaction mixture, said reaction mixture comprising:

(a) a complex of a first primer extension product and a second primer extension product, wherein the first primer extension product is generated by extension of a first primer hybridized to the template DNA with a DNA polymerase, wherein the first primer is a hybridizable to a multiplicity of template polynucleotide sites, wherein the first primer is a population of different composite primers each comprising an RNA portion and a 3' DNA portion, wherein each composite primer comprises a 3' random sequence, and wherein each composite primer is a tailed primer that comprises a 5' portion that is not hybridizable to the template DNA under conditions in which the first primer hybridizes to the template DNA, and wherein the second primer extension product is generated by extension of a second primer hybridized to the first primer extension product;

(b) an amplification primer, wherein the amplification is a composite primer comprising an RNA portion and a 3' DNA portion, wherein the amplification primer comprises some of the sequence of the first primer, wherein the first primer and the amplification primer are different primers, and wherein the amplification primer is hybridizable to the second primer extension product;

(c) a DNA-dependent DNA polymerase; and

(d) an agent that cleaves RNA from an RNA/DNA heteroduplex;

wherein the incubation is under conditions that permit RNA cleavage, primer hybridization, primer extension, and displacement of the first primer extension product from the second primer extension product when its RNA is cleaved and another amplification primer binds and is extended, such that primer extension and strand displacement are repeated, whereby multiple copies of an amplification product are generated.

66. (Canceled).

67. (Previously Presented): The method of claim 65, wherein said agent that cleaves RNA from a RNA/DNA heteroduplex is an enzyme.

68. (Previously Presented): The method of claim 67, wherein the enzyme that cleaves RNA from a RNA/DNA heteroduplex is RNase H.

69. (Previously Presented): The method of claim 67, wherein said DNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are the same enzyme.

70. (Previously Presented): The method of claim 67, wherein said DNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are different enzymes.

71.-73. (Canceled).

74. (Previously Presented): The method of claim 65, wherein the RNA portion of the amplification primer is 5' with respect to the 3' DNA portion.

75. (Original): The method of claim 74, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.

76. (Previously Presented): The method of claim 65, wherein the RNA portion of the amplification primer consists of about 7 to about 50 nucleotides.

77. (Original): The method of claim 76, wherein the DNA portion of the amplification primer consists of about 5 to about 20 nucleotides.

78. (Previously Presented): The method of claim 65, wherein the RNA portion of the amplification primer consists of about 10 to about 50 nucleotides.

79. (Original): The method of claim 78, wherein the DNA portion of the amplification primer consists of about 7 to about 20 nucleotides.

80. (Canceled).

81. (Original): The method of claim 65, wherein the reaction mixture further comprises a non-canonical nucleotide.

82. (Original): The method of claim 81, wherein the non-canonical nucleotide is dUTP.

83. (Original): The method of claim 65, wherein the reaction mixture further comprises a labeled nucleotide.

84.-107. (Canceled).

108. (Previously Presented): A method for amplification of a template DNA, comprising:

(a) randomly priming a template DNA with a first primer, wherein said first primer is hybridizable to a multiplicity of template polynucleotide sites, wherein the first primer is a population of different composite primers each comprising an RNA portion and a 3' DNA portion, and wherein each composite primer is a tailed primer that comprises a 5' portion that is not hybridizable to the template DNA under conditions in which the first primer hybridizes to the template DNA;

(b) extending said first primer with a DNA polymerase to generate a first primer extension product;

(c) hybridizing a second primer to the first primer extension product;

(d) extending said second primer with a DNA-dependent DNA polymerase and an RNA-dependent polymerase to generate a second primer extension product, whereby a complex comprising an RNA/DNA heteroduplex is generated;

(e) cleaving RNA from the first primer with an agent that cleaves RNA from a RNA/DNA heteroduplex;

(f) hybridizing an amplification primer to the second primer extension product, wherein said amplification primer is a composite primer comprising a RNA portion and a 3' DNA portion, wherein the amplification primer comprises some of the sequence of the first primer, and wherein the first primer and the amplification primer are different primers;

(g) extending the hybridized amplification primer by strand displacement DNA synthesis;

(h) cleaving RNA from the amplification primer with an agent that cleaves RNA from a RNA/DNA heteroduplex, such that another amplification primer hybridizes and is extended,

whereby multiple copies of an amplification product are generated.

109.-114. (Canceled).

115. (Previously Presented): The method of claim 108, wherein the agent that cleaves RNA from an RNA/DNA heteroduplex is an enzyme.

116. (Previously Presented): The method of claim 115, wherein the enzyme that cleaves RNA from an RNA/DNA heteroduplex is RNase H.

117.-119. (Canceled).

120. (Original): The method of claim 108, wherein the DNA polymerase of step (b) is a DNA-dependent DNA polymerase.

121. (Canceled).

122. (Previously Presented): The method of claim 108, wherein the DNA-dependent DNA polymerase and the RNA-dependent DNA polymerase are the same enzyme.

123. (Previously Presented): The method of claim 108, wherein the DNA-dependent DNA polymerase and the RNA-dependent DNA polymerase are different enzymes.

124.-126. (Canceled).

127. (Previously Presented): The method of claim 108, wherein the RNA portion of the first primer is 5' with respect to the 3' DNA portion.

128. (Previously Presented): The method of claim 108, wherein the RNA portion of the amplification primer is 5' with respect to the 3' DNA portion.

129. (Original): The method of claim 128, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.

130. (Original): The method of claim 127, wherein the 5' RNA portion of the first primer is adjacent to the 3' DNA portion.

131.-134. (Canceled).

135. (Previously Presented): The method of claim 108, wherein the RNA portion of the first primer consists of about 7 to about 50 nucleotides.

136. (Original): The method of claim 135, wherein the DNA portion of the first primer consists of about 5 to about 20 nucleotides.

137.-138. (Canceled).

139. (Previously Presented): The method of claim 108, wherein the RNA portion of the amplification primer consists of about 7 to about 50 nucleotides.

140. (Previously Presented): The method of claim 139, wherein the DNA portion of the amplification primer consists of about 5 to about 20 nucleotides.

141.-145. (Canceled).

146. (Previously Presented): The method of claim 108, wherein step (g) is carried out in the presence of a non-canonical nucleotide.

147. (Original): The method of claim 146, wherein the non-canonical nucleotide is dUTP.

148. (Previously Presented): The method of claim 108, wherein step (g) is carried out in the presence of a labeled nucleotide.

149.-190. (Canceled).

191. (Previously Presented): The method of claim 49, wherein the RNA portion of the first primer is 5' with respect to the 3'-DNA portion.

192. (Previously Presented): The method of claim 191, wherein the 5' RNA portion of the first primer is adjacent to the 3' DNA portion.

193. (Previously Presented): The method of claim 49, wherein said first primer and said second primer are the same primer.

194. (Previously Presented): The method of claim 193, wherein said first primer and said second primer are different primers.

195. (Previously Presented): The method of claim 65, wherein the RNA portion of the first primer is 5' with respect to the 3'-DNA portion.

196. (Previously Presented): The method of claim 195, wherein the 5' RNA portion of the first primer is adjacent to the 3' DNA portion.

197. (Previously Presented): The method of claim 65, wherein said first primer and said second primer are the same primer.

198. (Previously Presented): The method of claim 197, wherein said first primer and said second primer are different primers.

199. (Currently Amended): The method of any of claims 1, 49, 65, 84, or 108, ~~or~~ 149, wherein said template DNA is genomic DNA.

200. (Currently Amended): The method of claim 199, wherein said genomic DNA comprises a ~~multiplicity~~ multiplicity of genomic DNA templates.